IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re United States Patent Application of:		**Customer No.:**	**23448**
Applicant:	**DeVico, et al,.**	**Docket No.:**	**4115-144**
Application No.:	**09/684,026**	**Examiner:**	**U. Winkler**
Filed:	**October 6, 2000**	**Art Unit:**	**1648**
Title:	**VIRUS COAT PROTEIN/RECEPTOR CHIMERAS AND METHODS OF USE**	**Confirmation No.:**	**3193**

EXPRESS MAIL CERTIFICATE

I hereby certify that I am mailing the attached documents to the Commissioner for Patents on the date specified, in an envelope addressed to the Commissioner for Patents, Mail Stop RCE, P. O. Box 1450, Alexandria, VA, 22313-1450, and Express Mailed under the provisions of 37 CFR 1.10.

Candace White

Candace White

December 30, 2003

Date

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DECLARATION OF DR. ANTHONY L. DEVICO UNDER 35 U.S.C. §1.132 IN U. S. PATENT APPLICATION NO. 09/684,026

Mail Stop RCE
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Anthony L. DeVico hereby declare:

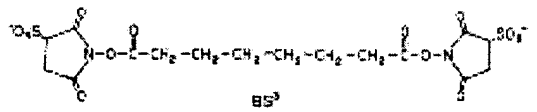
1. THAT I am a named co-inventor of the invention that is described and claimed in U.S. Patent Application No. 09/684,026 filed in the United States Patent and Trademark Office on October 6, 2000 in the names of Anthony L. DeVico, Timothy R. Fouts and Robert G. Tuskan for "VIRUS COAT PROTEIN/RECEPTOR CHIMERAS AND METHODS OF USE" (the "Application").

2. THAT, I am aware that the Application has been examined by the United States Patent and Trademark Office, that I have read the October 14, 2003 Advisory Action and the July 14, 2003 Office Action issued by the United States Patent and Trademark Office wherein the claims of the Application have been rejected on various grounds including the disclosures of U. S. Patent No. 5,843,454 (hereinafter DeVico '454), U. S. Patent No. 5,518,723 (hereinafter DeVico '723) and Chackerian, et al. (Proceedings of the National Academy of Sciences, March 1999).

3. That the present invention is described as chimeric polypeptides comprising a virus coat polypeptide sequence and viral receptor polypeptide sequence that are linked by an amino acid spacer, wherein the spacer is positioned between the virus coat polypeptide and the viral receptor polypeptide and linked thereto to form a single chain polypeptide. The amino acid sequence is of a sufficient length to allow folding of the single chain polypeptide to form an intramolecular interacting complex between the virus coat polypeptide and the viral receptor polypeptide.

4. THAT, as a co-inventor of the present application and cited references U.S. Patent Nos. 5,518,723 and 5,843,454, I am uniquely situated to identify the difference between my own prior work, as embodied in the cited DeVico '723 and '454 references, and the claimed invention of the instant application. Initially it should be noted that the complexes of DeVico '723 and '454 are ***covalently*** cross-linked. Steps were taken to permanently (covalently) bond the two molecules together. As stated in the DeVico '723 and DeVico '454 references, at column 4, lines 16-21 and column 4, lines 46-52, respectively, "We used a covalently linked gp120-CD4 complex as an immunogen. gp120 molecules were covalently coupled to soluble recombinant CD4 using bivalent cross-linking agent to ensure that the integrity of the complexes were maintained during any manipulation."

5. THAT, it is important to note that a crosslinking agent was used to covalently bond the gp120 and CD4 in the complex formation of both conjugates described in DeVico '723 and DeVico '454. The crosslinking agent was bis-sulfosuccinimidyl suberate, a homobifunctional cross-linking reagent with amine reactivity having a structure as set forth below:

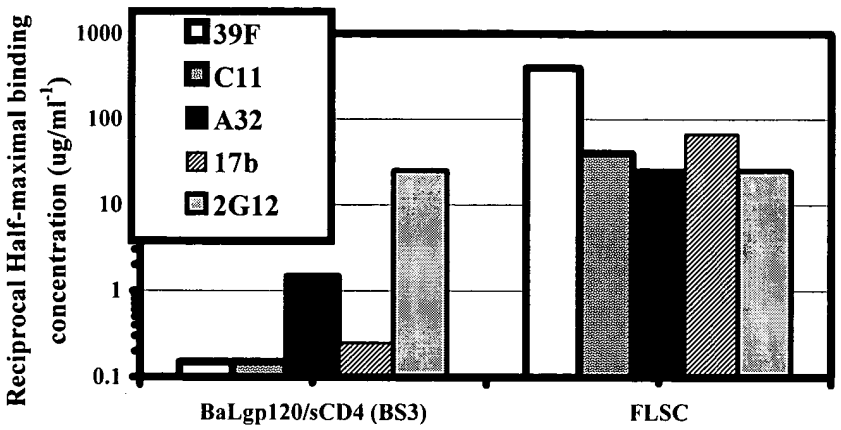


This crosslinking agent couples to molelcules containing primary amines by amide bonds and is used for preparation of receptor-ligand conjugates. However, use of this linker does not link two proteins in the binding scheme of a single chain length of amino acid residues as in the presently claimed invention but instead the crosslinking agent binds only to primary amines on the respective proteins. Thus, any formed complex is not a single chain polypeptide wherein the spacer forms peptide bonds between the terminal α-amino group of one protein and the terminal α-carboxyl group of protein.

6. THAT, according to the Examiner, "It remains the Offices position that substituting one type of spacer for another type of spacer is obvious absent a showing that the spacers achieve a complex formation that is not equivalent." In response to this contention, the following results show that the covalent crosslinking of BaLgp120/sCD4 complexes occludes epitopes that are exposed on the full length single chain (FLSC) chimeras of the presently claimed invention, and thus the covalently crosslinked complexes of DeVico '723 and '454 are not the equivalent of the FLSC of present invention. The differences between the complexes were shown by capturing FLSC and complexes of BaLgp120 and sCD4 onto D7324-coated ELISA plates. D7324 is a sheep polyclonal IgG that is reactive to the C-terminal region of gp120 and is an antibody that is commonly used to examine the antigenicity of HIV-1 envelope proteins by capture-ELISA. The BaLgp120/sCD4 complexes were then crosslinked for 30 mins with 0.5 mM Bis(sulfosuccinimidyl)suberate (Pierce), and then treated with 10 mM Tris-HCL to stop the reaction. Covalently bonded BaLgp120/sCD4 complexes & FLSC plates were then washed with TBS. Monoclonal antibodies against the V3 loop (39F), C1-C5 (C11), C1-C4 (A32), co-receptor binding domain (17b), and C3-V4 (2G12) regions of BaLgp120 were titrated onto the captured antigens. Bound antibodies were detected with goat-anti-human IgG labeled with horse-radish peroxidase.

As shown by the results set forth in the below figure, the covalent crosslinking reaction alters the structure of the BaLgp120/sCD4 complex and reduces the antigenicity of the 39F, C11, A32 and 17b epitopes by essentially occluding these epitopes. In contrast, all of these epitopes were exposed on the FLSC. Clearly, the covalent crosslinker alters the antigenic site and certainly impacts the function of these epitopes. For instance, the epitope recognized by 17b interacts with the R5 coreceptor. Occlusion of this epitope by the covalent crosslinker reduces the ability of the covalently crosslinked complex to interact with the coreceptor.

Covalent crosslinking of BaLgp120/sCD4 complexes occludes epitopes that are exposed on FLSC



However, the FLSC exposed these epitopes and showed a strong affinity for 17b. This unexpected result indicates that the covalently crosslinked complexes of the DeVico '723 and '454 references are not the equivalent of the FLSC polypeptide of the presently claimed invention. The FLSC polypeptide of the present invention provides for a far superior complex that can be used to potentially block HIV-1 via its coreceptor CCR5. Further, the FLSC polypeptide can be used to screen for reagents that may potentially block HIV-1 via its coreceptor CCR5.

7. THAT, according to the Examiner, the term "covalently bonded" was not defined in the specifications of either DeVico '723 or '454, and thus, the term was given its plain meaning in the art, wherein a bond shares electrons. Clearly, the complexes of both DeVico '723 and '454 are fused together by a covalent bond. I was involved in the prosecution of both the DeVico '723 and '454 patents and the terms "covalently bonded" were included in the claims because we formed the complex and maintained this complex by covalently linking the gp120 to the CD4 protein by using the chemical crosslinker. The chemical crosslinker was included to insure the integrity of the complex during immunization, antigen uptake and presentation to the immunized hosts. We demonstrated in both DeVico '723 and '454 that this covalently linked complex withstood the harsh environment of SDS PAGE. The outcome was entirely different when complexes formed through affinity binding were exposed to the denaturing environment of SDS PAGE because a formed covalent bond is not inherent in affinity bonding between CD4 and gp120 and a covalent bond does not naturally occur when you mix CD4 and gp120 together.

Instead, the intermolecular interacting complex of the present invention that is formed between the virus coat peptide (gp120) and viral receptor peptide (CD4) is due to a natural attraction. The relevant data comes from thermodynamic analysis and the crystal structure of the gp120-CD4 complex. Thermodynamic studies indicate that free gp120 is a disordered molecule that continually "samples" conformations in solution. CD4 binding "induces" a more ordered gp120 conformation. The interaction between gp120 and CD4 involves main-chain and side chain atoms. The "hot spot" on gp120 is a deep, roughly spherical pocket that accommodates phe 43 in the CD4 CDR2 loop. The pocket is lined with highly conserved residues. Direct interatomic contacts between CD4 and gp120 involve some CD4 residues and 26 gp120 residues. These interatomic contacts include approximately 219 Van der Waals contacts and 12 hydrogen bonds. The Phe 43 (on CD4) engages in hydrophobic interactions with a glu, trp, gly and ile in the gp120 pocket as does a CD4 arg with a gp120 val. Thus, the interacting complex formed between the gp120 and CD4 peptides linked by a foldable amino acid spacer is due to natural affinity.

9. THAT, in conclusion, the chimeras of the present invention are not disclosed or suggested in DeVico '723 or '454. Further, the covalently bonded complexes of both the cited references are different from the chimeras of the present invention for numerous reasons. Specifically, the process of crosslinking with the crosslinking agent occludes certain epitopes that are exposed in the presently claimed chimeras. Further, the covalently bonded complexes of DeVico '723 and '454 formed by a crosslinking agent were able to withstand the harsh denaturing environment of SDS PAGE while complexes formed through affinity binding are not able to withstand such conditions. Still further, the chimeras of the present invention are a single chain polypeptide that includes both the viral protein and the receptor protein in a single chain and separated therefrom by an amino acid spacer, and thus, peptide bonds are formed between the amino acid spacer and both proteins. Neither of the cited references, DeVico '723 or '454, teach or suggest a single chain polypeptide chimera. In fact, both cited references discourage one skilled in the art from going in the direction of the currently pending claimed invention because of the discussed shortcomings of any receptor-ligand complex formed strictly through affinity bonding.

As a below-named declarant, I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements, and the like, so made are punishable by fine or imprisonment, or both, under Section 1001 or Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued therefrom.

ANTHONY L. DEVICO, PHD

12/24/2003
DATE